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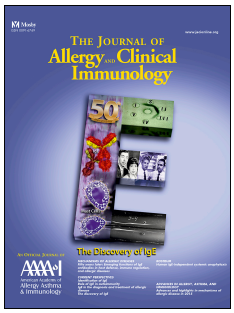

Serum autoreactivity predicts time to response to omalizumab therapy in chronic spontaneous urticaria

Janine Gericke, PhD, Martin Metz, MD, Tatevik Ohanyan, MD, Karsten Weller, MD, Sabine Altrichter, MD, Per Stahl Skov, MD, Sidsel Falkencrone, PhD, Janko Brand, PhD, Arno Kromminga, PhD, Tomasz Hawro, MD, Martin K. Church, PhD, DSc, Marcus Maurer, MD

PII: S0091-6749(16)31282-9

DOI: 10.1016/j.jaci.2016.07.047

Reference: YMAI 12437

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 9 February 2016

Revised Date: 10 June 2016

Accepted Date: 26 July 2016

Please cite this article as: Gericke J, Metz M, Ohanyan T, Weller K, Altrichter S, Skov PS, Falkencrone S, Brand J, Kromminga A, Hawro T, Church MK, Maurer M, Serum autoreactivity predicts time to response to omalizumab therapy in chronic spontaneous urticaria, *Journal of Allergy and Clinical Immunology* (2016), doi: 10.1016/j.jaci.2016.07.047.

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Serum autoreactivity predicts time to response to omalizumab therapy in chronic spontaneous urticaria

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Clinical Implications:

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This study suggests that a positive basophil histamine release assay (BHRA) is predictive of a slow response to omalizumab in CSU patients while a negative BHRA predicts a rapid response.

Capsule summary:

A slow response to omalizumab (anti-IgE) in CSU patients is strongly correlated with IgG-anti-FcεRI-mediated serum-induced basophil histamine release suggesting that, in these patients, effects on FcεRI are critical for the mechanism of action of omalizumab.

Key words:

Chronic spontaneous urticaria; omalizumab; basophil histamine release; ASST; IgG-anti-FcεRI: mast cell.

Abbreviations:

ASST, autologous serum skin test; BHRA, basophil histamine release assay; CSU, chronic spontaneous urticaria; UAS7, 7-day urticaria activity score.

To the Editor

Omalizumab (anti-IgE) has been shown to be effective in chronic spontaneous urticaria (CSU) in a number of clinical trials.[1] However, in clinical practice, some CSU patients are fast responders to their first injection of omalizumab, often within days, while others are slow responders with therapy taking weeks to be effective. This letter examines the possible underlying mechanisms.

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Sixty-four CSU patients (46 females) whose symptoms were not controlled with H_1-antihistamines up to four times the recommended dose were treated with omalizumab at the Charité Department of Dermatology. Their median age was 47 (range 23 – 85) years and the median duration of their CSU was 3 years (range 4 months – 40 years). Seven patients had received omalizumab in the past but not for at least 6 weeks prior to enrolment in the study, and their symptoms had returned in full. All analyses were performed according to the declaration of Helsinki and approval was obtained from the Charité ethics committee (EA1/268/13). All patients gave signed informed consent.

Omalizumab 300 mg was injected subcutaneously three times at four-week intervals. The first day of response was defined as the first of seven continuous days when the UAS7[2] was ≤ 6. Venous blood was taken before the first omalizumab treatment and centrifuged at 2,500 rpm for 10 minutes.

By the end of week 12, 56 (88%) had responded to omalizumab, and 8 (12%) were non-responders (Figure 1A). Of the 56 responders, 39 patients (70%) responded within 8 days (fast responders), 18 (32%) within the first day. Seventeen patients (30%) responded between 8 days and 3 months (slow responders). The cut-off time of 8 days between fast and slow responders was chosen as it corresponds with the peak plasma level of omalizumab after its initial injection.[3]

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We hypothesized that a slow response to omalizumab occurs in CSU patients in whom IgG antibodies to unoccupied IgE receptors (FcεRI) activate mast cell mediator release to cause wheal and angioedema formation.[4] This hypothesis is based on the knowledge that omalizumab first complexes soluble IgE then sequesters IgE released from mast cells thus uncovering membrane FcεRI, which subsequently decays slowly over several weeks.[5]

To test this, the basophil histamine release assay (BHRA) was used. This assay may be used to detect serum autoantibodies directed against either the cell bound IgE or unoccupied FcεRI. In this study, basophils were stripped of their IgE to assess FcεRI only. The BHRA was done as previously described (Supplement 1).[6] Analysis of the omalizumab responders showed that most BHRA-positive patients responded only after the second injection (Figure 1B), with a median time to response of 29 days, whereas BHRA negative patients had a median time to response of only 2 days (Figure 1C). Furthermore, only one of the 39 fast responders was BHRA positive, whereas 8 of the 17 slow responders were BHRA positive (*P*=0.0001, Fishers exact test; Figure 1D).

The hypothesis was also tested using the autologous serum skin test (ASST) [7] in which 50 µl of fresh undiluted autologous serum were injected intradermally into the volar forearm. Similar volumes of 0.9% NaCl saline and 100 µg/ml histamine were used as negative and positive controls. The ASST was taken to be positive when the serum-induced wheal had a diameter at least 1.5 mm greater than the saline-induced wheal at 30 minutes. For clinical reasons, the ASST was performed on 51 patients only. Twelve of the 33 fast

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responders were ASST positive, while 10 of the 13 slow responders showed a positive ASST (*P* = 0.012, Fishers exact test, Figure 1E, F).

There were no statistical differences between fast and slow omalizumab responders with respect to age, body mass index, or disease activity or duration. Similarly, pretreatment IgE levels, neutralization rates of IgE, and free IgE and omalizumab levels post treatment were not significantly different between slow and fast responders to omalizumab (Table 1).

When comparing BHRA with ASST, it must be realized that BHRA is more specific in that it was designed to identify serum IgG antibodies to FcεRI. However, it is time consuming and may not be available in all clinics. In contrast, the ASST is easy to perform clinically but is less specific, because it will also detect IgG antibodies against mast cell-bound IgE, and may contain other histamine releasing factors that can lead to a positive ASST response.[7] Even so, there was a significant relationship between BHRA and ASST results (Spearman's rho = 0.448, $P < 0.01$). All BHRA positive patients were ASST positive. BHRA-positive and ASST-positive omalizumab responders are 4.5 and 5.5 times more likely to have a slow response to treatment compared with BHRA-negative and ASST-negative responders, the relative risks with 95% confidence limits were 4.54 for BHRA (2.42 - 8.53, z-test $P < 0.001$) and for ASST 5.50 (1.37 - 22.11), z-test $P < 0.05$.

The strength of this study is the highly significant correlation between the length of time to the onset of activity of omalizumab and BHRA, strongly

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suggesting that a positive BHRA may predict a slow response to omalizumab. Limitations of the study are that all of the data are derived from an open observational study without controls. A further weakness is that the story is not entirely black and white. For example, one patient with positive BHRA and ASST became symptom free within one day of the first omalizumab injection. Also, there were nine BHRA negative patients in the slow responder group. However, five of these did have a positive ASST. These apparent anomalies suggest that in some patients, CSU may have more than one mechanism.[8]

In conclusion, there are significant correlations between a positive BHRA and ASST and the time to symptom relief with omalizumab. The fact that a positive BHRA is predictive of a slow response to omalizumab suggests Omalizumab works via reducing FcεRI expression in these patients. Larger prospective studies are needed to confirm these preliminary observations.

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Figure 1. Characteristics of the response to omalizumab treatment in patients with chronic spontaneous urticaria.

(A) The percentage of responders (UAS7 ≤ 6) plotted against the day of response. The blue arrows indicate the days of omalizumab injections. **(B-F)** ASST-positive and BHRA-positive omalizumab responders show slower onset of effects. **(B**, **E)** Kaplan-Meier curves showing proportion of patients with positive and negative basophil histamine release assay (BHRA; **B**) and positive and negative autologous serum skin test (ASST; **E**) achieving response to therapy over time. **(C**, **F)** Box-whiskers plots presenting median, interquartile range, maximum and minimum as well as individual dots of day to therapy response in patients with positive and negative BHRA (**C**) and ASST (**F**). **(D)**, Release of histamine in cell supernatants expressed as percentage of total histamine from donor cells in fast (≤ 8 days) and slow (< 8 days) responders to omalizumab. The black solid vertical lines indicate median values and the dotted horizontal line indicates cut-off for positive values of basophil histamine release assay (BHRA). As negative control, the buffy coat cells responded to a mixture of healthy sera with less than 5%. As a positive control we used anti-IgE, resulting in 45% release. Statistical analyses were performed using Kaplan-Meier estimator and log-rank statistics (**B**, **E**) or Mann-Whitney U test (**C**, **D**, **F**).

Table 1

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		All complete responders (n=56	complete response within 8 days	complete response after 8 days	p-value
Age		48 (33-60)	49 (37-58)	42 (31-63)	0.544
Sex	female	40 (71.4%)	28 (71.8%)	12 (70.6%)	0.583
	male	16 (28.6%)	11 (28.2%)	5 (29.4%)	
BMI		27.3 ±4.8	27.9 ±4.9	26.1 ±4.6	0.192
UAS7[1]		24.0 ±9.7	23.0 ±9.3	26.5 ±4.6	0.215
Disease duration[1]		36 (16-102)	40 (18-103)	24 (14-85)	0.240
ASST+[1]		23/46 (50.0%)	12/33 (36.4%)	11/13 (84.6%)	**<0.01**
BHRA+[1]		9/55 (16.4%)	1/38 (2.6%)	8/17 (47.1%)	**<0.001**
anti-FcεRI+[1]		5/44 (11.4%)	4/30 (13.3%)	1/14 (7.1%)	0.485
anti-IgE+[1]		1/44 (2.3%)	1/30 (3.3%)	0/14 (0.0%)	0.682
total IgE[1]		205.4 ±229.6	239.2 ±250.6	130.0 ±155.0	0.104
Free IgE after omalizumab[2]		31.8 ±47.7	31.2 ±39.6	33.2 ±64.7	0.893
% IgE neutralization[2]		94.7 ±13.4	96.7 ±2.2	90.0 ±24.5	0.106
Omalizumab (µg/ml)[2]		16.2 ±7.8	16.6 ±7.2	15.2 ±9.2	0.557

Table 1. CSU responders to omalizumab: Demographic, clinical and laboratory characteristics. Abbreviations: ASST, autologous serum skin test; BHRA, basophil histamine release assay; BMI, body mass index; UAS7, 7-day urticaria activity score. Data are given as n (%) for sex, ASST, BHRA; median (IQR) for age and duration of disease (in months); or mean ±SD for BMI, UAS7, total IgE, free IgE after omalizumab, % neutralization, omalizumab. Statistical differences between responder groups in age, ASST+,

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BHRA+, anti-FcεRI+, and anti-IgE+ were analyzed using Chi2 or Fisher's exact test when appropriate. Statistical differences between responder groups in BMI, UAS7, and total IgE were analyzed using unpaired t test.[1]Before start of omalizumab treatment; [2]four weeks after start of omalizumab treatment. Note that not all of the patients were tested for ASST and/or BHRA, because of various reasons including time constraints, availability of personnel to do the serum preparations and injections and patient consent.

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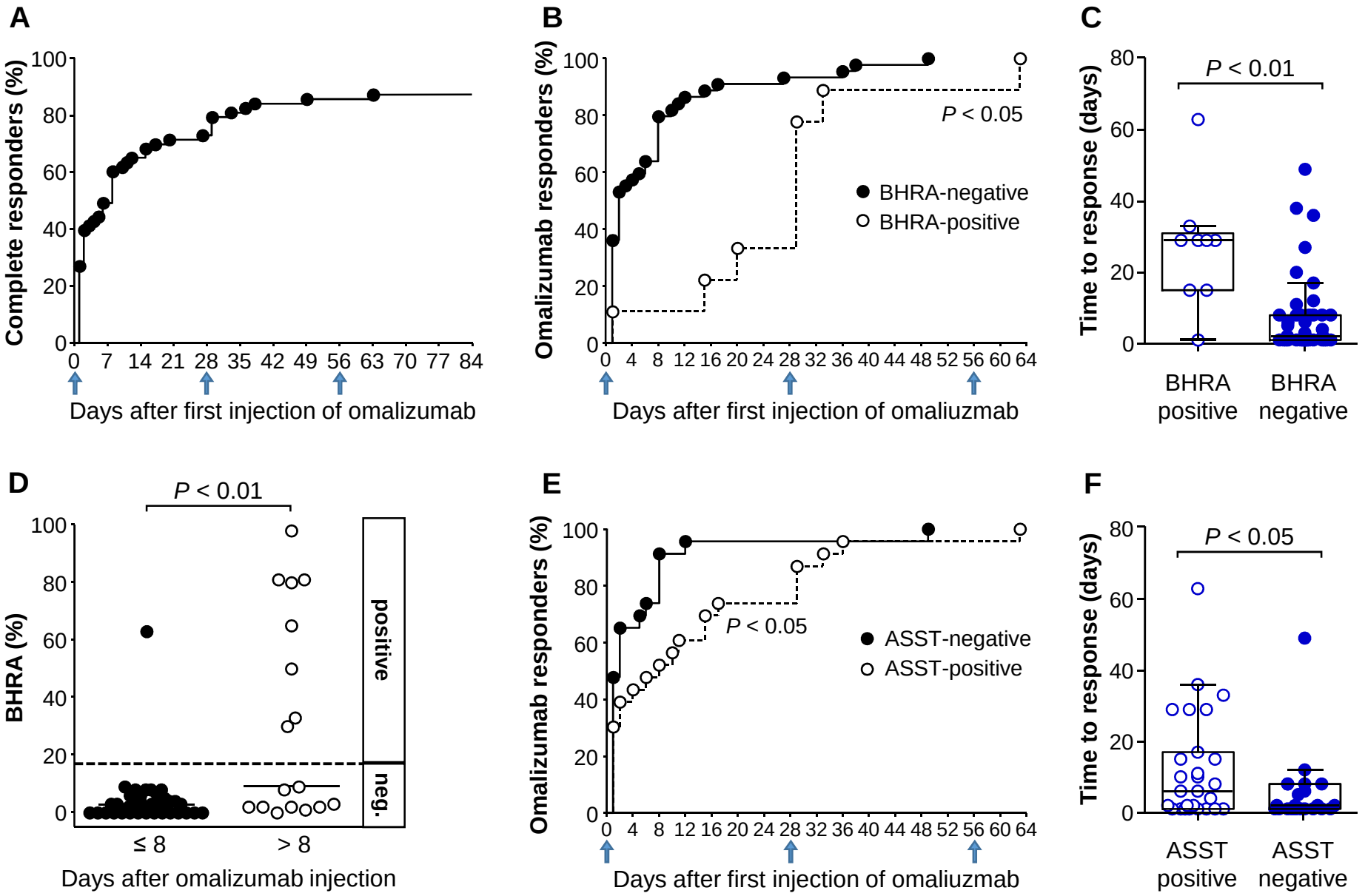
A
Complete responders (%)
100
80
60
40
20
0
0 7 14 21 28 35 42 49 56 63 70 77 84
Days after first injection of omalizumab
B
Omalizumab responders (%)
P < 0.05
BHRA-negative
BHRA-positive
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64
Days after first injection of omaliuzmab
C
Time to response (days)
P < 0.01
BHRA positive
BHRA negative
D
BHRA (%)
P < 0.01
positive
neg.
≤ 8
> 8
Days after omalizumab injection
E
Omalizumab responders (%)
P < 0.05
ASST-negative
ASST-positive
Days after first injection of omaliuzmab
F
Time to response (days)
P < 0.05
ASST positive
ASST negative

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Material and Methods

Autologous serum skin test (ASST)

The ASST was performed as previously described.[S1] Briefly, venous blood was withdrawn before the first administration of omalizumab. Samples were centrifuged at 2,500 rpm for 10 minutes and the serum separated. Fresh serum was used for the ASST or stored at -80℃ for the serum-induced basophil histamine release (BHRA) and other analyses.

A volume of 50 µl of fresh undiluted autologous serum was injected intracutaneously into the volar surface of the forearm. A similar volume of 0.9% NaCl saline was injected as negative control, and histamine (50 µl of 100 µg/ml, i.c. or skin prick test) was used simultaneously as positive control. Wheal and flare responses were measured at 30 minutes. The ASST response was taken to be positive when the serum-induced wheal had a diameter at least 1.5 mm greater than the saline-induced wheal.

Serum induced basophil histamine release assay (BHRA)

The serum-induced basophil histamine release assay may be used to detect autoantibodies in patient sera directed against either the cell bound IgE or against free IgE receptors (FcεRI). In this study, basophils were stripped of their IgE in order to assess serum antibodies to FcεRI.

Basophils were prepared and histamine release assessed as previously described.[S2] Briefly, blood bank buffy coats containing 1-2%

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basophils were obtained from fresh healthy donor blood. As cell responses vary between donor cells, four blood bank buffy coats were combined. The buffy coats were mixed with equal volumes of RPMI containing IL-3 in a final concentration of 1 ng/mL, and the samples were stored overnight at 2-8 ℃. The next day, the buffy coats were centrifuged to remove plasma and RPMI. Thereafter, the samples were washed with physiological saline, exposed to low pH using a stripping buffer (pH 3.6 from RefLab, Copenhagen, Denmark) to remove IgE from the basophils, and resuspended in Pipes buffer (RefLab, Copenhagen, Denmark) before incubation with patient sera. Each serum was tested at dilutions of 20% and 10%, data is shown using 20% dilutions.

Total histamine content was determined after cell lysis with 100 µl of 7% $HClO_4$ for 60 minutes at 37℃. After centrifugation, 25 µl supernatants were transferred to glass fiber coated microtiter plates and histamine was measured according to RefLab (Copenhagen, Denmark) instructions. Triplicate histamine determinations of each sample were performed. Assay variation was < 7%. Assay sensitivity was 5 ng histamine/ml. The release of histamine in cell supernatants was expressed as the percentage of total histamine content from lysed donor cells.

BHRA was considered positive if histamine levels were >16.5% spontaneous. As negative control, the buffy coat cells responded to a mixture of healthy sera with less than 5%. As a positive control we used anti-IgE, resulting in 45% release. The mean ± SEM histamine release of the BHRA negative patients was 2.8 ± 0.4% and that of the BHRA positive patients 63 ± 8%.

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Total serum IgE, anti-IgE and anti-FcεRI

Serum levels of total IgE, IgG-anti-IgE and IgG-anti-FcεRI were determined as previously described.[S3] Briefly, recombinant FcεRI was fractionated by 14% reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDSPAGE) and transferred to nitrocellulose. Blots were blocked for 1 h in 1% (v/v) Tween-20 in PBS buffer (154 m*M* NaCl, 20 m*M* KH_2PO_4, pH 7.6). For detection of autoantibodies, sera were diluted 100-fold in PBS. Monoclonal antibody directed to the N-terminal tag $RGSH_4$ (Qiagen) was diluted 2,000fold in PBS. After a 12-hour incubation at room temperature with diluted sera, the blots were washed and incubated with rabbit anti-human IgG or rabbit anti-mouse (Jackson Immunoresearch Laboratories, West Grove, Pa., USA) conjugated with alkaline phosphatase. The NBT/BCIP staining system was used as chromogenic substrate.

Quantification of free serum IgE, IgE neutralization and omalizumab concentrations

A commercially available recoveryELISA kit was used for the quantification of free IgE, IgE neutralization rates and omalizumab levels in patient serum (BioTeZ Berlin Buch GmbH, Berlin, Germany) as described previously. [S4]

Supplementary references

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